THE INFLUENCE OF TEMPERATURE ON SYNAPSIS IN HYBRID SALIVARY GLANDS [1]

ELISABETH GOLDSCHMIDT

Department of Zoology, Hebrew University, Jerusalem, Israel

The giant chromosomes of Diptera are the most favorable material for studies of the extent and nature of synaptic association. The mitotic and meiotic chromosomes in this group are so small that they hardly offer a clear picture of the details of their conjugation during prophase or interphase. It has become customary, therefore, to rely on salivary glands for studies of synaptic association in structural hybrids of Drosophila, although it is not known whether the synapsis of giant chromosomes reflects with any degree of accuracy the mode of association of homologues in meiosis or mitosis. While the conjugation of homologues is believed to be a prerequisite for the occurrence of crossing-over, the observed variations in cross-over frequency and their relationship to the phenomenon of synapsis are not understood. It seems desirable, therefore, to determine whether synaptic association varies with environmental factors, which are known to influence the frequency of crossing-over. Beginning with Plough (1917), much attention has been paid to the effect of temperature on crossing-over. It was for this reason that temperature was chosen as the varying factor in the present study.

The question of variation in synapsis, apart from its possible bearing on crossing-over, gains additional interest in the light of the "Structural Theory" of the position effect mechanism (Ephrussi and Sutton, 1944; Gersh and Ephrussi, 1946).

Variation in the extent of synapsis with age and temperature was observed in salivary glands of Chironomus hybrids (Goldschmidt, 1947). Synapsis was found to decrease with rising age of the larvae. In larvae grown at high (30° C.) and low (13° C.) temperatures, there was less synapsis than in larvae reared at moderate temperatures (20–25° C.). While salivary glands of Chironomus offer material for smears during an extended prepupal period, the suitable stage for smearing in Drosophila is of very short duration. This precise definition of the physiological age suitable for smearing should tend to minimize any possible age effect on synapsis in preparations obtained from this organism and should render them a suitable object for the study of the temperature effect.

Materials and Methods

The effect of temperature on synapsis was examined in two structural hybrids in the genus Drosophila. As an instance of a small inversion within a species, the

[1] This work was carried out in the Zoology Department, University of California, Berkeley, during the tenure of the Mary E. Woolley International Fellowship of the American Association of University Women, awarded by the International Federation of University Women.

I am greatly obliged to Dr. C. Stern for his generous help and advice, and to Dr. Aloha M. Hannah for many valuable suggestions.

Arrowhead inversion in Chromosome III of *D. pseudoobscura* was chosen. For a large inversion in a species hybrid, the condition of Chromosome III R in the *D. melanogaster* × *simulans* hybrid was studied.

For the *D. pseudoobscura* crosses, 10 females and 10 males were mated in half-pint bottles and allowed to oviposit for three days at room temperature. The parents were then removed and the bottles transferred to constant temperature chambers of 13, 17.5, 20, 25 and 28° C. (this being the upper limit for larval development in this species).

The *D. melanogaster* × *simulans* cross was obtained by the method described by Uphoff (1949), 10 *melanogaster* females and 15 *simulans* males being mated (without etherizing) in "creamers." If the cross was found to be fertile, the parents were removed to half-pint bottles and allowed to oviposit for two days, at room temperature. After removal of the parents, the bottles were kept at 13, 17.5, 20, 25, 29 and 31° C. Although the food employed was molasses-cornmeal-agar enriched with yeast, "yeasting" of the bottles at the time of appearance of second instar larvae was found indispensable for obtaining good preparations of salivary chromosomes.

The glands were dissected, at room temperature, in 45% acetic acid, stained in aceto-carmine and made permanent according to a method of Dr. A. M. Hannah, by transferring through alcohol and xylol into Canada Balsam, without removing the cover-slip, whenever possible.

The preparations examined for the *pseudoobscura* cross include male and female offspring of reciprocal matings between Standard and Arrowhead. The *melanogaster* × *simulans* preparations were all from female larvae of the cross employing *melanogaster* as the female parent.

The following arbitrary system of recording asynapsis was adopted: *D. pseudoobscura.* The loop extends from band 70B to 76B in Chromosome III (Dobzhansky, 1944).

	Asynaptic units
Loop completely unsynapsed	3
Loop synapsed except for sections 70B–D and/or 76A–B	1
Loop synapsed less than this	2
Complete asynapsis in section before loop (63A–70A)	1
Any degree of asynapsis in section before loop	½
Complete asynapsis in section after loop (76C–81D)	1
Any degree of asynapsis in section after loop	½

D. melanogaster × *simulans.* The loop (figured by Pätau, 1936) extends from band 84F to 93E.

	Asynaptic units
Loop completely unsynapsed	4
Loop three-quarters unsynapsed	3
Loop half unsynapsed	2
Loop one-quarter unsynapsed	1
Loop less than one-quarter unsynapsed	½
Section before loop (81F–84F) completely unsynapsed	1
Section before loop partly unsynapsed	½
Section after loop (93F–100F) completely unsynapsed	3
Section after loop two-thirds unsynapsed	2
Section after loop one-third unsynapsed	1
Section after loop less than one-third unsynapsed	½

In order to avoid unconscious selection, the labels of all preparations were covered with non-transparent paper before scoring. Twenty nuclei were scored in each preparation of the *pseudoobscura* cross and ten nuclei in each of the *melanogaster* × *simulans* crosses.

Results

a) Arrowhead × Standard inversion

The results are presented in Table I and Figure 1. There was more asynapsis at 13° C. on one hand and at 28° C. on the other than at intermediate temperatures (17.5–25° C). Although the standard errors are large, the difference between 13° C. and 17.5° C. and the difference between 25° C. and 28° C. are significant. The slight rise in asynapsis between 17.5° C. and 20° C. and the decline from 20° C. to 25° C. are not significant. It may be concluded that synapsis in this inversion is comparatively most complete at intermediate temperatures. The large standard errors reflect, of course, the considerable differences existing between different individuals grown at the same temperature. Moreover, as is known to every worker with salivary gland chromosomes, the variation between nuclei of the same gland is also pronounced. Nevertheless, a cursory inspection of a preparation was usually sufficient to determine whether the larva had grown at an extreme or at an intermediate temperature.

Table I

Asynaptic units in Arrowhead-Standard inversion

Temp. °C.	No. of larvae	Mean asynaptic units	Standard error	*t*	*p*
13	15	30.2	±7.3		
				3.2	0.01–0.001
17.5	14	22.5	±5.8		
				0.7	0.6 –0.5
20	13	23.7	±3.2		
				1.3	0.3 –0.2
25	14	21.3	±6.0		
				4.3	<0.001
28	13	30.0	±4.3		

b) Melanogaster × simulans inversion

The results are summarized in Table II and Figure 1. Synapsis is most complete at 13° C. There is less pairing at 17.5° C. Asynapsis falls off from 17.5° C. to 25° C. and then rises again until 31° C. The differences between 13° C. and 17.5° C., 17.5° C. and 25° C. and between 25° C. and 31° C. are significant.

It should be mentioned that the preparations of the 13° C. series could usually be assigned to this group without reading the label. Although they do not show significantly more synapsis than those of the 25° C. group, their chromosomes are peculiar, because the fusion of the homologues in synapsed regions is usually less intimate than in any other series. Since under the present system of scoring any mode of contact between homologues was recorded as "synapsis," the particularly loose synapsis of the 13° C. group is not expressed in Table II.

TABLE II

Asynaptic units in melanogaster × simulans inversion

Temp. °C.	No. of larvae	Mean asynaptic units	Standard error	*t*	*p*
13	22	15.4	±5.2		
				3.3	0.01–0.001
17.5	15	22.6	±8.0		
				0.7	0.5 –0.4
20	13	20.5	±7.2		
				1.4	0.2 –0.1
25	17	17.3	±5.3		
				1.4	0.2 –0.1
29	17	19.6	±4.5		
				2.4	0.05 –0.02
31	10	23.8	±4.3		

Significance of difference between:

Temperatures ° C.		
13 and 25	1.1	0.3 –0.2
17.5 and 25	2.2	0.05–0.02
25 and 31	3.3	0.01–0.001
13 and 31	4.5	<0.001

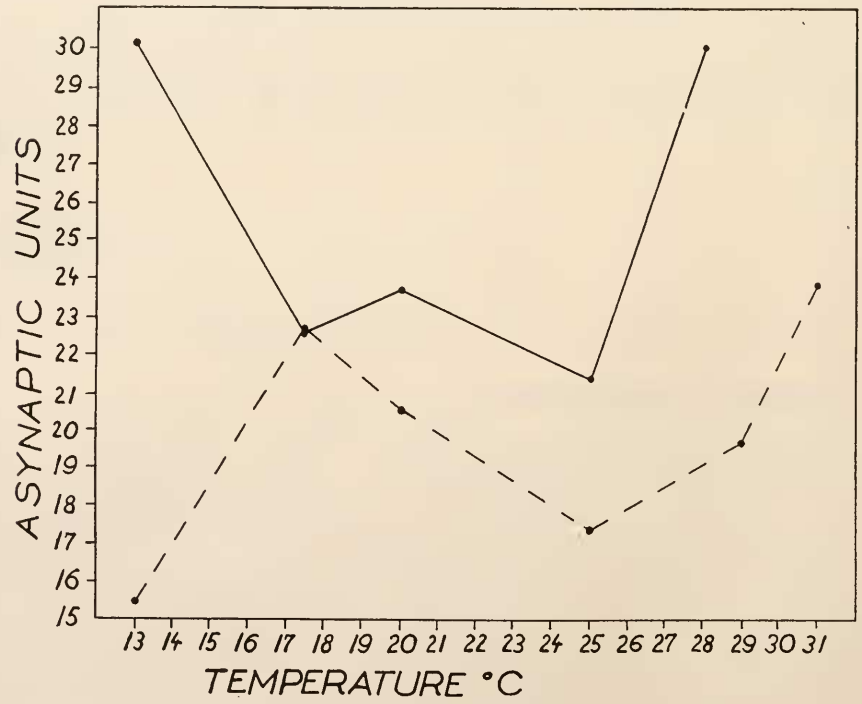

FIGURE 1. Variation with temperature of asynaptic units. Heavy line: Arrowhead × Standard inversion in *D. pseudoobscura*. Broken line: *D. melanogaster* × *simulans* hybrids.

Discussion

Since all the results are based on smears, it might be doubted whether they reflect the true association of homologues in intact nuclei. Hinton (1945) showed that terminal associations are not easily broken, even when stretched with a micromanipulator. This observation makes it unlikely that truly synapsed regions should be separated by the pressure exerted in smearing.

In the two graphs presented in Figure 1, the common features are the minimum of asynapsis at moderate temperatures and the rises on either side of the intermediate range. The most outstanding difference lies in the presence of a second minimum of asynapsis in the *melanogaster* × *simulans* inversion and its absence in the Arrowhead × Standard cross. It has been mentioned that at 13° C., synapsis in the *melanogaster* × *simulans* hybrid is extensive, but not intimate. This phenomenon renders a strict comparison of the two graphs at the 13° C. level impossible.

The presence of a minimum of asynapsis at moderate temperatures in the two rearrangements investigated confirms the findings in Chironomus (Goldschmidt, 1947). However, in view of the considerable differences existing, especially between the *melanogaster* × *simulans* cross and the other two, the combined data do not justify the assumption that we are dealing with a phenomenon universal in structural hybrids. It should be remembered, moreover, that the results obtained with structural hybrids may not have any direct bearing on the process of synapsis in structurally homozygous individuals.

Caution in evaluating the present data seems especially indicated in consideration of the complex evidence regarding the influence of temperature on crossing-over and on chiasma frequency. Plough (1917, 1921) found a minimum of crossing-over at moderate temperatures in the autosomes of *D. melanogaster,* but could not confirm this effect for the X-chromosome in regions distant from the centromere. Stern (1926) found a temperature effect in the X-chromosome near the spindle attachment (from garnet to bobbed). Clark (1943) did not detect any influence of temperature on crossing-over in Habrobracon. White (1934) obtained a curve similar to that of Plough for chiasma frequency in Stenobothrus, but his graphs for Locusta and Schistocerca differ markedly from this pattern. The situation is further complicated by the data of Stern and Rentschler (1936) on somatic crossing-over in the X-chromosome of *D. melanogaster.* This is high at both 17 and 25° C. and low at 30° C.

Much significance has been attributed to the resemblance of Plough's curve to the variation of plasma viscosity with temperature (Heilbrunn, 1928; Frey-Wyssling, 1948). The temperature-viscosity curves of some organisms exhibit several maxima and minima within the biological temperature range. If the absence of a simple linear relationship to temperature may indeed be considered as a common characteristic, this is certainly shared by the graphs presented here. The minimum of crossing-over at intermediate temperatures is paralleled by a minimum of asynapsis in a similar temperature range: a paradoxical situation in view of the fact that chromosomes presumably do not cross-over unless they are previously paired. This paradox might resolve itself if local repulsions between paired homologues proved to be involved in the mechanism of crossing-over.

The influence of temperature on various position effects, previously established by Gowen and Gay (1933, 1934) and various later workers, was studied more recently by Hinton (1949) and Gersh (1949). Their results indicate that with a number of position effects, extreme temperatures produce either smaller or larger effects than moderate temperatures. Since synapsis in some structural hybrids is similarly affected by temperature, it seems possible that some position effects of the variegated type may indeed be connected with synapsis variations, setting up different stresses in the chains of gene proteins.

Summary

1. The effect of temperature on synapsis was studied in salivary glands of *Drosophila pseudoobscura* heterozygous for the Arrowhead inversion, and of *D. melanogaster* × *simulans* hybrids.

2. In the *pseudoobscura* larvae, significantly less asynapsis was observed at 17.5, 20 and 25° C. than at extreme temperatures (13 and 28° C.).

3. In the *melanogaster* × *simulans* hybrids, synapsis was most complete at 13 and at 25° C., and there were two significant peaks of asynapsis at 17.5 and at 31° C.

4. The common feature of the two distributions is the minimum of asynapsis at moderate (20 and 25° C.) temperatures. The possible relationships of this result with the known temperature effects on crossing over and on the position effect are discussed.

LITERATURE CITED

Clark, A. M., 1943. Linkage relations in Habrobracon and the effects of temperature, X-ray and age on crossing-over. *Proc. Pennsylvania Acad. Sci.*, **17**: 47–64.

Dobzhansky, Th., 1944. Chromosomal races in *Drosophila pseudoobscura* and *Drosophila persimilis*. *Carn. Inst. Wash. Publ., 554;* pp. 47–144.

Ephrussi, B., and E. Sutton, 1944. A reconsideration of the mechanism of position effect. *Proc. Nat. Acad. Sci.*, **30**: 183–197.

Frey-Wyssling, A., 1948. Submicroscopic morphology of protoplasm and its derivatives. Elsevier Publ. Co., New York, Amsterdam.

Gersh, E. S., 1949. Influence of temperature on the expression of position effects in the Scute-8 stock of *Drosophila melanogaster* and its relation to heterochromatization. *Genetics,* **34**: 701–707.

Gersh, E. S., and B. Ephrussi, 1946. The mechanism of position effect. Experiments on the phenotypic expression of position effects in relation to changes in pairing of neighbouring chromosome regions. *Proc. Nat. Acad. Sci.*, **32**: 87–94.

Goldschmidt, E., 1947. Studies on the synapsis in salivary chromosomes of hybrid Chironomus larvae. *J. Genet.*, **48**: 194–205.

Gowen, J. W., and E. H. Gay, 1933. Effect of temperature on eversporting eye-color in *Drosophila melanogaster*. *Science,* **77**: 312.

Gowen, J. W., and E. H. Gay, 1934. Chromosome constitution and behavior in eversporting and mottling in *Drosophila melanogaster*. *Genetics,* **19**: 189–208.

Heilbrunn, L. V., 1928. The colloid chemistry of protoplasm. Protoplasmamonographien, 1. Borntraeger, Berlin.

Hinton, T., 1945. A study of chromosome ends in salivary gland nuclei of Drosophila. *Biol. Bull.,* **88**: 144–165.

Hinton, T., 1949. The modification of the expression of a position effect. *Amer. Nat.,* **83**: 69–94.

Pätau, K., 1936. Chromosomenmorphologie bei *Drosophila melanogaster* und *Drosophila simulans* und ihre genetische Bedeutung. *Naturwiss.,* **23**: 537–543.

PLOUGH, H. H., 1917. The effect of temperature on crossing-over in Drosophila. *J. Exp. Zool.*, **24**: 147–210.

PLOUGH, H. H., 1921. Further studies on the effect of temperature on crossing-over. *J. Exp. Zool.*, **32**: 187–202.

STERN, C., 1926. An effect of temperature and age on crossing-over in the first chromosome of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.*, **12**: 530–532.

STERN, C., AND V. RENTSCHLER, 1936. The effect of temperature on the frequency of somatic crossing-over in Drosophila. *Proc. Nat. Acad. Sci.*, **22**: 451–453.

UPHOFF, D. E., 1949. The expression of alleles at the cubitus interruptus locus in hybrids between *Drosophila melanogaster* and *Drosophila simulans*. *Genetics*, **34**: 315–326.

WHITE, M. J. D., 1934. The influence of temperature on chiasma frequency. *J. Genet.*, **29**: 203–215.